REMARKS

Claims 87 and 89 – 98 are pending. New claims 99 and 100 have been added. Support for claim 99 is found in the specification at page 8, lines 2 – 11, and at page 25, lines 21 – 26. Support for claim 100 is found in the specification at page 27, lines 27 – 35.

Claims 87-97 have been provisionally rejected on the ground of non-statutory obviousness-type double patenting as being unpatentable over claims 1-8 of copending Application No. 10/969,646.

Applicant respectfully submits that a terminal disclaimer will be filed when allowable subject matter is indicated.

Claims 87-97 have been rejected under 35 U.S.C. 112, first paragraph as failing to comply with the enablement requirement.

The Applicant has discovered that contacting a somatic cell nucleus with a cytostatic factor cytoplasm (e.g. meiotic metaphase II (MII) oocyte cytoplasm or a cytostatic factor cytoplasm (CSF) extract prepared from eggs arrested in MII) followed by an activating egg cytoplasm (e.g. an oocyte just prior to S-phase or an activating egg extract prepared from eggs in the cell cycle just prior to the S-phase) reprograms the nucleus to allow development of an embryo and a cloned animal (i.e. by eliminating the somatic cell patterns of gene structure and function such as methylation patterns).

The Examiner asserts that the specification does not reasonably provide enablement for incubation of a nucleus in an MII or induced [activated] egg, development of an embryo and indicators of activation other than nucleus swelling, nucleic acid replication and entry into mitosis. Applicant respectfully disagrees for the following reasons.

The Applicant has disclosed in the specification the *exemplary* use of egg extracts prepared from the cytoplasm of non-induced eggs arrested in meiotic metaphase II (CSF extracts) and from the cytoplasm of an induced egg (activated egg extracts). Applicant respectfully submits that a skilled artisan understands that exposure of a somatic nuclei to these

defined egg extracts, which the applicant has discovered results in reprogramming of somatic cell nuclei, is equivalent to exposure of somatic nuclei to the cytoplasm of intact eggs that are in the same defined state. That is, cytoplasmic extracts prepared from eggs arrested in MII is equivalent to the cytoplasm of an intact egg that is arrested in MII and cytoplasmic extracts prepared from eggs that are in the cell cycle just prior to S-phase is equivalent to the cytoplasm in an intact egg that has been activated to enter the S-phase of the cell cycle. Applicant respectfully submits that cytoplasmic extract from eggs arrested in MII have the same factors as the cytoplasm in an intact egg that is arrested in MII, e.g. components that trigger chromatin condensation and nuclear membrane breakdown, as this is what happens during MII.

Furthermore, the Applicant has clearly defined the state of the cell cycle in an *oocyte cytoplasm* required for activation. See for example, the specification at page 25, lines 21-26, which states:

> "It appears that when an egg is at the point in the cell cycle just prior to the S-phase, the egg cytoplasm is most active in supporting activation, and as the egg proceeds into and past the S-phase, it appears to produce material inhibitory to nuclear activation" (Specification, page 25, lines 21-26).

Thus, a skilled artisan would understand by reading the instant specification that exposure of somatic cell nuclei to the cytoplasm of an intact egg in these defined states would reprogram the somatic nuclei just as the exemplified cytoplasmic extracts did. Moreover, it is easy to screen for a reprogrammed cell by looking at the cellular replication that occurs because the nucleus is transplanted from one cell to another. Accordingly, undue experimentation is not required.

The Examiner asserts that from the specification, the disclosed method requires two incubations for reprogramming; incubating a somatic nuclei in an extract containing a cytostatic factor (e.g. cytostatic factor cytoplasm (CSF) extract prepared from eggs arrested in MII) and an "activating" extract. The Examiner states that "the use of both extracts is required by the disclosure" (office action, page 4, lines 17-18). Applicants respectfully submit that the instant claims reflect two incubations; contacting somatic nuclei with the cytoplasm of an MII oocyte followed by an activating egg cytoplasm.

The Examiner asserts that the specification does not reasonably provide enablement for development of an embryo and indicators of activation other than nucleus swelling, nucleic acid replication and entry into mitosis. The Examiner asserts that while the specification discloses activation of several nuclei as shown by nuclear swelling, nucleic acid replication and entry into mitosis, there is no indication that cytokinesis occurred to produce a multicellular embryo. Applicant respectfully submits that specification teaches that contacting a somatic cell nucleus with a cytostatic factor cytoplasm (e.g. meiotic metaphase II (MII) oocyte cytoplasm or a cytostatic factor cytoplasm (CSF) extract prepared from eggs arrested in MII) followed by an activating egg cytoplasm (e.g. an oocyte just prior to S-phase or an activating egg extract prepared from eggs in the cell cycle just prior to the S-phase) reprograms somatic cell nuclei in a sufficient manner to allow development of a cloned animal. The Applicant shows that their method results in 1) nuclear swelling, 2) *complete* nucleic acid replication and 3) entry into mitosis. Applicant respectfully submits that a showing of *complete* nucleic acid replication and entry into mitosis is a sufficient showing that successful reprogramming has occurred and that the cells will continue to divide to create a multicellular embryo.

Evidence of complete nucleic acid replication is found in the specification at page 68, Example 6, lines 24-36:

> "Furthermore, it appears that replication of the entire genome was achieved"

and at page 69, lines 24-27:

> "First mitosis occurred relatively early and was not accompanied by DNA fragmentation. These observations are consistent with the view that genome replication was complete in this experiment"; Example 10, page 73, lines 33-35 "After 150 minutes of incubation in fresh activating extract DNA replication was complete and the nuclei entered mitosis"

At the time of filing the Applicant recognized that a difficulty in cloning somatic cell nuclei from a mammalian species is that the somatic nuclei are imprinted DNA methylation patterns of gene structure and function and that reprogramming eliminates methylation patterns of the somatic cells (See specification, page 58, lines 35 to pg 60 line 9). Applicants respectfully submit that complete genome replication can *not* occur in the absence of eliminating imprinted DNA methylation patterns. The Applicant, in the instant specification, teaches how to successfully

achieve *complete* genome replication and entry of the cells into mitosis. Applicant respectfully submits that this confirms that successful reprogramming has occurred and further submit that a somatic cell that has been successfully reprogrammed can proceed to divide (cytokinesis) and direct development of a multicellular embryo.

The Examiner asserts that the specification does not teach what contacting means. Applicant respectfully submits that an skilled artesian understands that "*contacting*" a nucleus with, means "*exposing*" a nucleus to, whether it be by incubation with or by any other means. The term is well known in the art and need not be clarified in the specification. Applicant discloses in the Specification page 6, lines 12-14:

> "Activation is brought about by contacting a pretreated, or preferably a further pretreated nucleus with an activating extract";

and at page 10 lines 12-15:

> "prior to being treated with the activating extract, the pretreated nuclei are further pretreated by contact with a CSF extract [e.g. a cytostatic factor cytoplasm (CSF) extract prepared from eggs arrested in MII] …"

Terms that are well known to those skilled in the art need not be defined in the specification.

Based on the above Applicant respectfully submits that the specification provides guidance and enables an artisan to make and use the invention as claimed and requests that the rejection of claims 87-97 35 U.S.C. 112, first paragraph be withdrawn.

Claims 97-98 have been rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement.

The Examiner asserts that at the time of filing the art recognized that nuclear transfer or cloning to produce a term animal was unpredictable and states:

> "Even if applicant's method results in a reprogrammed somatic nucleus, it is documented in the arena of nuclear transfer/cloning that pregnancy does not necessarily mean live births." (office action page 5, lines 8-10).

Applicant respectfully submits that the inefficiency of maintaining pregnancy to the point of live birth is *inherent* to nuclear transfer methods and that the level of efficiency or unpredictability

associated with the claimed methods is well within the range of acceptable to those practicing in the art of the invention. As will be explained below, low levels of efficiency should not be an issue. One can readily take the recipient egg and screen for those developing into a proper blastocyst. That blastocyst is then used for implantation. This is the standard technique used at the time of filing for *in vitro* fertilization – and the skilled artisan would be aware of such techniques. As is known in the art, obtaining a viable blastocyst has substantially lower odds than implanting a viable blastocyst into the animal, and this step can readily be screened. Thus, the issue is not that the odds are low – but that the art confirms that following these steps will result in a viable non-human clone.

The Applicant has discovered a method for reprogramming somatic nuclei that allows for successful cloning of whole animals. The Applicant's method involves contacting a somatic nucleus with an MII oocyte cytoplasm followed by contacting the nucleus with an activated egg cytoplasm. The Applicant shows that their method results in nuclear swelling, *complete* nucleic acid replication and entry into mitosis. As indicated above, Applicant respectfully submits that this is a sufficient showing that successful reprogramming has occurred and that the cells can continue to divide to create a multicellular embryo. The Applicant's studies were originally performed in an effort to *reduce* the unpredictability of the reprogramming step and thus improve and control the efficiency of cloning, and they have successfully done so. The Applicant has provided a showing that reprogramming in fact occurs. Applicant respectfully submits that, even if cytokenesis and development of an embryo only occurs in a finite number of cases of those reprogrammed cells (statistically speaking), the Applicant has still shown that their method leads to successful reprogramming that can lead to live birth, animal clones. The level of efficiency or unpredictability associated with the claimed methods that can lead to successful cloning is a level of efficiency and unpredictability that is acceptable to those practicing the art of cloning.

The successful use by others in using a mitotic MII cytoplasm followed by an activating cytoplasm for cloning of whole animals confirms that the instant specification provides an enabling methodology for cloning of live animals. In response to the Office Action dated March 23, 2006, Applicant submitted the following references: Sullivan et al. Biol. Of Reprod. 70:146-153 (2004); Polejaeva et al., Nature, 407: 86-90 (2000); Betthauser et al., Nature Biotechnology 18: a005-59 (2000); and Chesne et al., Nature 20: 366-369 (2002).

Sullivan et al. confirm that it is possible to successfully remodel a somatic nucleus in a cell extract and *produce live offspring* (page 152, column 1, paragraph 4, lines 1-3) using the methodology of the applicants invention. Sullivan et al. show that exposure of somatic nuclei, *in vitro,* to a reprogramming extract from human cells arrested in MII results in reprogramming; disassembly of somatic nuclei, condensed chromatin, and removal of nuclear components (page 149, column 1, paragraph 1, lines 1-5). As stated on page 51, column 1, lines 11-14):

> "Remodeling of the somatic chromatin was demonstrated by induction of condensation of chromosomes in the mitotic extract."

Sullivan et al. then transferred the condensed chromatin to an enucleated oocyte prior to activation of the oocyte with calcium ionophore (page 146, column 2, paragraph 3, lines 4-8). Exposure of the remodeled somatic chromatin to an activated egg cytoplasm resulted in formation of viable blastocysts *in vitro* in 661 out of 5880 cases (page 150, column 1, para. 3, line 2 and continued at column 2, line 1). Furthermore, when the embryos were transferred to a recipient female calf, the embryos developed to term resulting in live births in 42 of the 273 recipients (page 150, column 2, lines 7-11).

The Examiner asserts that Sullivan et al. uses extracts from MBDK cells whereas the specification only discloses using an MII egg extract to activate donor nuclei and thus does not provide enablement (office action, page 9, para. 1). Applicant respectfully submits that an MII extract of egg is a sufficient showing that an MII extract from MDBK cells would work for purposes of reprogramming and visa versa. The reason being that the cell cycle stage of metaphase II is similar in egg cells (meiosis) and somatic cells (mitosis) and a skilled artisan would expect that extracts from either egg cells or somatic cells would have the similar factors with equivalent function and the same general effect on chromosmes (e.g. factors that support nuclear envelope breakdown and chromosome condensation such as cytostatic factor (CSF) and mitosis promoting factor (MPF)). The Applicant teaches on page 10 of the specification lines, 31-35, and continued on page 11, lines 1-4, that CSF extracts (e.g. eggs arrested in meiotic metaphase II, i.e. MII extract) contain factors which aid in nuclear activation, such as mitosis promoting factor (MPF) and cytostatic factor (CSF) and that MPF may help bring about activation of chromosomes by stimulating chromosome condensation. The specification further

teaches at page 35, lines 1-5, that CSF and MPF are factors present in CSF extract which are believed to aid in subsequent activation of quiescent nuclei by altering cytoskeletal proteins, nuclear matrix protein, and nuclear histones, particularly by phosphorylation of these proteins.

Others in the art, besides Sullivan et al. have confirmed that somatic nuclear cell transfer is almost completely dependent on the use of a MII cytoplasm of a cell arrested in meiotic metaphase or mitotic metaphase or unactivated egg cytoplasm (cytoplasms described in the instant application at page 10, 35-36), and that the use of MII cytoplasm in nuclear transfer methodology followed by exposure of nuclei to activating cytoplasm results in *live births.*

Polejaeva et al., has used dual nuclear transfer technology to generated cloned pigs. The technology involves first exposing somatic nuclei to non-activating oocyte cytoplasm in MII (donor cells fused to enucleated MII oocytes, page 8, column 1, lines 31-33) and subsequently exposing the nuclei to activating oocyte cytoplasm (page 87, column 2, lines 16-17), to an activated oocyte, followed by nuclear transfer to an enucleated *in vivo* produced zygote. Poleva et al. even states on page 88 colum 2, lines 9-19:

> "It has been suggested that the use of MII oocytes may improve 'reprogramming' of the donor genetic material owing to the occurrence of nuclear envelope breakdown and premature chromosome condensation, thus exposing the donor chromatin to maternally derived oocyte factors involved in early development. To take advantage of this here, we used MII oocyts as cytoplast recipients for the first nuclear transfer embryo reconstruction."

Applicants respectfully submit that the instant specification provides the first disclosure to show that exposure of somatic nuclei to MII cytoplasm (i.e. cytoplasm form a cell arrested in meiotic metaphase, or mitotic metaphase) results in reprogramming of somatic nuclei sufficient for cloning.

The Examiner asserts that Polejaeva et al. does not provide enablement because Polejaeva et al. uses intact oocytes or intact zygotes as the incubating cytoplasm and the Applicant's specification only contemplates the use of extracts to reprogram/activate somatic cell nuclei (office action, page 9, para. 3). Applicant respectfully submits that the disclosure in the specification of the use of egg extracts was *exemplary*. Applicant respectfully reiterates that a skilled artisan understands that exposure of a somatic nuclei to the egg extracts defined in the

instant specification, which the applicant has discovered results in reprogramming of somatic cell nuclei, is equivalent to exposure of somatic nuclei to the cytoplasm of intact eggs that are in the same defined state. That is, cytoplasmic extracts prepared from eggs arrested in MII is equivalent to the cytoplasm of an intact egg that is arrested in MII and cytoplasmic extracts prepared from eggs that are in the cell cycle just prior to S-phase is equivalent to the cytoplasm in an intact egg that has been activated to enter the S-phase of the cell cycle. Applicant respectfully submits that cytoplasmic extract from eggs arrested in MII have the same factors as the cytoplasm in an intact egg that is arrested in MII, e.g. components that trigger chromatin condensation and nuclear membrane breakdown, as this is what happens during MII. Accordingly, Polejaeva et al. *does* provide confirmation of enablement of the Applicants invention, that is contacting somatic nuclei with a non-activating oocyte cytoplasm in MII followed by contacting the nuclei with an activating cytoplasm results in successful reprogramming sufficient for live birth cloning.

Enablement is further supported by the success of Betthauser et al. and Chesne et al. in cloning of pigs and rabbits when using methodology of the Applicants invention, i.e. contacting a somatic cell nucleus with a cytoplasm of an MII oocyte (e.g. meiotic metaphase II (MII) oocyte cytoplasm or a cytostatic factor cytoplasm (CSF) extract prepared from eggs arrested in MII) followed by an activating egg cytoplasm (e.g. cytoplasm of an oocyte just prior to S-phase or an activating egg extract prepared from eggs in the cell cycle just prior to the S-phase).

Betthauser et al., successfully coned pigs by exposing somatic nuclei to enucleated oocytes arrested in MII (fusion of donor cell to enucleated oocyte, see page 1058, paragraph 2, lines 7-10) followed activation of the oocytes (i.e. exposure to activating egg cytoplasm) with calcium inomycin and DMAP (page 1058, paragraph 3, lines 1-4).

Chesne et al. successfully clones rabbits by exposing somatic nuclei to MII ooplasm by fusion of donor cells to recipient enucleated MII oocytes (page 1, column 1, para. 2, lines 1-3). The transferred nuclei were then exposed to an activated cytoplasm through activation by electostimulation and exposure to cyclohexamide and DMAP(page 1, column 1, para. 2, lines 11-14). Activation in this manner stimulates the zygote to enter S-phase, exposing the somatic nuclei to cytoplasm in the cell cycle just prior to the S-phase.

The Applicant teaches reprogramming of somatic nuclei in cytoplasm of an MII oocyte followed by an activating egg cytoplasm and the use of this methodology to produce cloned animals (specification, page 58, lines 20-25). Applicant respectfully submits that the successful use by others (Sullivan et al.; Polejaeva et al.; Betthauser et al.; and Chesne et al.) in using a mitotic MII cytoplasm followed by an activating cytoplasm for cloning of live animals provides confirmation that the instant specification provides an enabling methodology for successful reprogramming and cloning of live birth animals. Further, it confirms that the level of efficiency or unpredictability associated with the claimed methods is well within the range of acceptable to those practicing in the art of the invention. Accordingly, any skilled artisan can follow the teachings of the instant specification to successfully produce cloned animals.

The Examiner asserts that the art is clear that reprogramming is essential for term birth of a cloned animal, and that incubation in Xenopus extracts is unpredictable in its ability to sufficiently reprogram a differentiated cell nucleus to support term development (Office action page 6, lines 21-24). The Examiner cites, with respect to reprogramming using Xenopus extracts such as disclosed in the specification, Hochedlinger at al.(Review article, *Nature*, 44: 1061-1066, 2006) as stating:

> "No stable reprogramming was seen in reversibly permeabilized somatic cells that were subsequently passaged in culture, suggesting that an intact oocyte might be required for functional de-differentiation." (page 1064, col.1, para. 4, lines 15-18).

Applicant respectfully submits that Hochedlinger et al., in the above passage, is referring to the work of Hansis et al. (Exhibit 1) and Hansis et al. **did not use extract prepared from eggs arrested in MII**. Accordingly, Hochedlinger at al./Hansis et al. does *not* teach that incubation in Xenopus extract prepared from eggs arrested in MII is unpredictable.

Applicant respectfully submits that the applicants disclosure of contacting a somatic nucleus with an MII oocyte extract (or cytoplasm) followed by contacting the nucleus with an activated egg extract (or cytoplasm) resulting in nuclear swelling, *complete* nucleic acid replication and entry into mitosis, is a sufficient showing that that successful reprogramming has occurred. A skill artisan understands from the Applicants disclosure that such successful reprogramming will lead to generation of a cloned multicellular embryo that can be carried to term for live birth.

The Examiner contends that the specification teaches treatment of permeabilized donor nuclei with two Xenopus egg extracts, however, there is no enablement for exposure directly to egg cytoplasm of any variety (office action page 7, para. 2). Applicant respectfully reiterates that a skilled artisan understands that exposure of a somatic nuclei to the egg extracts defined in the instant specification, which the applicant has discovered results in reprogramming of somatic cell nuclei, is equivalent to exposure of somatic nuclei to the cytoplasm of intact eggs that are in the same defined state.

With respect to claims 87, 97 and 98, the Examiner asserts that these claims encompass the production of a cloned mammal and it is noted that cross-species nuclear transfer is regarded as unpredictable for production of a cloned-animal. The Examiner cites Meirelles to provide evidence that cross species nuclear transfer is unpredictable for the production of a cloned animal. Applicant respectfully submits that claims 87, 97 and 98 recite "wherein the recipient egg is from the same species as the somatic cell nucleus." Accordingly, Applicant is not claiming cross-species nuclear transfer.

Accordingly, the specification provides guidance and enables an artisan to make and use the invention as claimed. Applicant respectfully requests that the rejection of claims 87-97 under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement be withdrawn.

Claims 87 and 89-98 have been rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement.

The Examiner asserts that the invention encompasses incubation of a somatic cell nucleus in an intact MII egg followed by incubation in an intact induced egg, however the Specification does not reveal that this invention was disclosed (Office action page 10, lines 3-7).

Applicant respectfully submits that the Applicants disclosure of incubating a somatic cell nucleus with a cytostatic factor extract (e.g. cytostatic factor cytoplasm (CSF) extract prepared from eggs arrested in meiotic metaphase II or activated eggs arrested in mitotic metaphase) followed by an activating egg extract (e.g. an activating egg extract prepared from eggs in the cell cycle just prior to the S-phase) (Specification, page 10, lines 12-15, and lines 32-36) exemplifies that cytoplasm of intact eggs in these defined states, also results in reprogramming

of somatic cell nuclei. As indicated above, a skilled artisan understands that exposure of a somatic nuclei to these defined egg extracts, which the applicant has discovered results in reprogramming of somatic cell nuclei, is equivalent to exposure of somatic nuclei to the cytoplasm of intact eggs that are in the same defined state. That is, cytoplasmic extracts prepared from eggs arrested in MII is equivalent to the cytoplasm of an intact egg that is arrested in MII and cytoplasmic extracts prepared from eggs that are in the cell cycle just prior to S-phase is equivalent to the cytoplasm in an intact egg that has been activated to enter the S-phase of the cell cycle. Applicant respectfully submits that cytoplasmic extract from eggs arrested in MII have the same factors as the cytoplasm in an intact egg that is arrested in MII, e.g. components that trigger chromatin condensation and nuclear membrane breakdown, as this is what happens during MII.

Furthermore, the Applicant has clearly defined the state of the cell cycle in an *oocyte cytoplasm* required for activation. See for example, the specification at page 25, lines 21-26, which states:

> "It appears that when an egg is at the point in the cell cycle just prior to the S-phase, the egg cytoplasm is most active in supporting activation, and as the egg proceeds into and past the S-phase, it appears to produce material inhibitory to nuclear activation" (Specification, page 25, lines 21-26).

Thus, a skilled artisan would understand by reading the instant specification that exposure of somatic cell nuclei to the cytoplasm of an intact egg in these defined states would reprogram the somatic nuclei just as the exemplified cytoplasmic extracts did.
As such, Applicant respectfully submits that the invention as claimed, claims 87 and 89-98, is disclosed in the specification, thus complying with the written description requirement. Applicants respectfully request that the rejection of claims 87 and 89-98 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement be withdrawn.

In view of the foregoing, applicant respectfully submits that all claims are in condition for allowance. Early and favorable action is requested.

FEE AUTHORIZATION

If any fee deficiencies are associated with this submission, the Commissioner is authorized to debit such deficiencies to our Deposit Account No. 50-0850. Any overpayments should be credited to said Deposit Account.

Date: February 4, 2008

Respectfully submitted,

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